International Journal of Agricultural Technology 2019 Vol. 15(1): 75-90
Available online http://www.ijat-aatsea.com
ISSN: 2630-0613 (Print) 2630-0192 (Online)

Effects of light intensity on the growth, photosynthesis and leaf microstructure of hydroponic cultivated spinach (*Spinacia oleracea* L.) under a combination of red and blue LEDs in house

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Nguyen, T. P. D., Tran, T. T. H. and Nguyen, Q. T. (2019). Effects of light intensity on the growth, photosynthesis and leaf microstructure of hydroponic cultivated spinach (*Spinacia oleracea* L.) under a combination of red and blue LEDs in house. International Journal of Agricultural Technology 15(1): 75-90.

Abstract The effect of four different light intensities (90, 140, 190 and 240 $\mu mol/m^2/s$) on the growth, photosynthesis and leaf microstructure of hydroponic cultivated spinach under a combination of red and blue LEDs (R660/B450 = 80/20) in house was investigated. The plant height, leaf number, leaf area, NGR, NAR, Chl*a*, Chl(*a* +*b*), photosynthetic capacity increased with increasing intensity. Althrough, there was not statistically significant difference in Chl(*a*+*b*) and carotenoid contents between the 190 and 240 $\mu mol/m^2/s$ treatments but they differed in Chl*a*. Furthermore, the leaf area, NGR, NAR, Chl*a* and Chl(*a*+*b*) contents were significantly higher in 190 $\mu mol/m^2/s$ treatment than 240 $\mu mol/m^2/s$ treatment. Differences in leaf thickness, palisade tissue length and spongy tissue length were statistically significant between 4 treatments. Even, leaf thickness in 190 $\mu mol/m^2/s$ treatment was found by 1.4 folds increased compare with 90 $\mu mol/m^2/s$ treatment. When light intensity increased, epidermal cell area, stomatal length, stomatal width increased. In the adaxial leaf surface and abaxial leaf surface, the epidermal cell area was highest in 190 $\mu mol/m^2/s$ treatment. But in all two leaf surfaces stomatal width was highest in the *240* $\mu mol/m^2/s$ treatment. The results showed that fresh weight and dry weight of stem and leaf, theoretical yeild, final harvest yeild were not highest in 240 $\mu mol/m^2/s$ treatment but in 190 $\mu mol/m^2/s$ treatment. Our results suggested that 190 $\mu mol/m^2/s$ light intensity may be appropriated the intensity for growth of spinach.

Keywords: carotenoid, Fv/Fm, leaf thickness, stomatal length, palisade tissue length

Introduction

Light is not only a major source of energy but it also is one of the environmental determinants for plant growth. The light intensity and quality are essential for the plant development, morphology and various physiological responses. Changes in light spectrum have strongly influenced on the leaf

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anatomy, morphology and physiology (Macedo *et al.,* 2011). In order to adjust to different light regimes of the environment, plants have developed many mechanisms including morphological and physiological changes at various levels (Zhang *et al.,* 2003; Fan *et al.,* 2013).

Low radiation intensity can lead to increase specific leaf area (SLA) and plant height. These adaptations aimed to maximize available light absorption for photosynthesis (Steinger *et al.,* 2003). Meanwhile, high radiation intensity is associated with many physiological and morphological characteristics that are appropriate to environmental conditions, such as reduced SLA to protect plants from high radiation exposure; increase leaf thickness by increasing the number of cell layers or increasing the development of palisade and spongy tissue. This modification helps to prevent or mitigate the damage caused by excessive illumination by light energy, ensuring good photosynthesis (Matos *et al.,* 2009). In plant tissues such as stems and leaves, the synthesis of secondary metabolites may change due to physiological, biochemical, and genetic factors in which light is one of the photoreceptors (Lefsrud *et al.,* 2008). On the other hand, according to Terashima *et al.* (2009) the light in the red and blue regions of the spectrum are mainly absorbed by photosynthetic pigments. About 90% absorption by plant leaves are blue or red light (Terashima *et al.,* 2009). Thus, photosynthetic rate, physiology and plant growth, development are significantly influenced by blue or red light (Chen *et al.,* 2014).

Nowadays, more and more studies about combination of red and blue light are carried out. The combination of red and blue light was an effective lighting source to plant development (Wheeler *et al.,* 1991), and promote the plant health (Nhut *et al.,* 2003). Additionally, the combination of red and blue in 1:1 ratio might promote fresh weight and dry weight in many plant species such as Lilium, Chrysanthemum and tomato (Lian *et al.,* 2002; Kim *et al.,* 2004; XiaoYing *et al.,* 2011). Indeed, green LEDs (400-500 nm) combined and red LEDs have the positive effect not only on growth and nutritional quality of green leafy vegetable, which has been reported in several studies. According to Goins (1997), wheat (*Triticum aestivum* L.) could grown normally under monochromic red LEDs, but became stronger with higher dry matter content and more seed numbers, when the red LED light was supplemental with a light blue color. Other researches indicated that the response of plants (growth, flowering time and secondary metabolites) to light quality was not the same in different species (Johkan *et al.,* 2012).

Green vegetables are one of the healthiest mineral and nutrient sources that we can grow. Some vegetables are also considered as functional foods, which are used as precious medicinal herbs to enhance health and prevent disease. However, vegetable qualities that are fresh and safe are of particular

concern. In response to the actual demand, the cultivation of vegetables in the new direction such as planting vegetables without soil, no irrigation, no need to use sunlight and build closed production model to control nutrition quality is an urgent necessity now essential.

Spinach (*Spinacia oleracea* L.) is one of the best leafy vegetables. Spinach is rich in vitamins A, K, D, E, omega-3 fatty acids and a variety of health benefits. Researchers were identified more than 10 different flavonoid compounds in spinach, which play important roles in anti-inflammatory and anticancer agents, because they slow down the division and decrease the number of cancer cell. Although, spinach is a vegetable in cold countries, which grows well at 18-20^{o}C (Boese and Huner, 1990). Moreover, spinach can survive in low light conditions but it is difficult to grow at high temperatures. Therefore, the research to be able to grow spinach in all season of year, especially in out of season crops in direction of urban and high-tech agriculture, to serve the demand for safe vegetables with high nutritional content is an urgent necessity in Vietnam. Information about the effects of different light intensities on the growth, photosynthesis and leaf microstructure of hydroponic cultivated spinach under a combination of red and blue LEDs allowed us to define a suitable light intensity to cultivate hydroponic spinach in an indoor system.

Materials and methods

Plant materials and growth condition

This experiment was conducted in air-conditioned houses at the Institute of Agrobiology, Vietnam National University of Agriculture (in 2018). The room temperature was maintained at 27^{o}C ± 0.2^{o}C and humidity was maintained at 62.5 % ± 0.5%.

Heat-treated F1 seeds PD512 of spinach (*Spinacia oleracea* L.) were provided by Phu Dien Trading & Production Company Limited. The seeds were cultivated in Klasmann TS-2 subtrate (product of Germany) in plastic trays (125 holes). When the seedlings had two true leaves, selected the plants with the same size then transplanted into plastic cage (7cm diameter, 10 cm height, 2 plants/cage), into the circulating hydroponic system. The experiment was conducted in 5 hydroponic systems racks, with 4 rigs/rack and 50 cm spacing between rigs. Each rig with 5 hydroponic solution tubes in parallel and 9 plants/1 tube, 45 plants/rig. Every hydroponic system rack was equipped with LED lighting at four light intensities: 90, 140, 190 and 240 μmol/m2/s (corresponding to a treatment, red and blue LEDs at ratio R660/B450 = 80/20).

The distance between plants was 15 cm. The LEDs were manufactured and supplied by Rang Dong Light Source & Vacuum Flask. The plants were grown under a 12-h light/12-h dark photoperiod. Harvest time was 40-50 days after sowing and repeated 5 times for each treatment.

Growth parameters

Leaf number, leaf area and plant height were counted and measured. Relative growth rate (RGR) is calculated using the following equation (Hoffmann *et al.*, 2002):

$$RGR = (\ln W_2 - \ln W_1) / (t_2 - t_1)$$

Net assimilation Rate (NAR) is calculated using the following equation (Radford, 1967):

$$NAR = [(W_2 - W_1) / (t_2 - t_1)] * [(\ln A_2 - \ln A_1)/(A_2 - A_1)]$$

Where:

ln= natural logarithm

t_1= time one (in days); W_1= Dry weight of plant at time one (in grams)

t_2= time two (in days); W_2= Dry weight of plant at time two (in grams)

A_1 = leaf area of plant at time one; A_2 = leaf area of plant at time two (in square meters)

Photosynthetic parameters and photosynthetic pigments

Net photosynthesis rate (Pn - µmol/m^2/s) and stomatal conductance (G*s*-mmol/m^2/s**)** were performed using TPS1 portable photosynthetic System Ver.1.2.1 (USA).

Chlorophyll a fluorescence

These measurements were carried using handheld Chlorophyll Fluorescence Meter, OS-30 (ADC, UK). Plant leaves were kept in the dark for 30 min and then exposed to the weak. The minimum fluorescence (F0), the maximum fluorescence (Fm) was measured by Fluorescence Meter. The variable fluorescence (Fv = Fm - F0), maximum quantum yield of photosystem (PS) II (Fv/Fm) and variable chlorophyll fluorescence ratio (Fv/F0) were calculated according to Van Kooten and Snel (1990) and Pereira *et al.* (2000).

Photosynthetic pigments

Chlorophyll was extracted from the leaves at a similar position within each treatment. Leaves were weighed out in 0.5 g quantities (fresh weight) and samples were grounded in mortars. The extractions were performed using 10 ml

(V) of 80% acetone. The mixtures were centrifuged at 6000 rpm for 30 minutes. The optical densities of extracts were measured with a spectrophotometer (Specto 2000RSP, Labomed, Inc. U.S.A) at 663 nm, 645 nm and 470 nm. The chlorophyll and carotenoid concentrations were determined by Arnon's method (1949):

Chla (g/L) = 0.0127 A_{663} – 0.00269 A_{645}

Chlb (g/L) = 0.02291 A_{645} – 0.00468 A_{663}

Chla+b (g/L) = 0.0202 A_{645} + 0.00802 A_{663}

Carotenoid (g/L) = (A_{470} – 0.00182 Chla – 0.08502 Chlb)/198

Then the pigment contents in the leaves are converted to mg/g

Anatomical features of leaf

The anatomical features of the mesophyll cells in the leaves of spinach were made out using Clark's method of (1981). Cross-sections were cut by hand. Leaf compactness was calculated using the following formula: Leaf compactness=Palisade tissue length/Leaf thickness. This thickness ratio of palisade to spongy tissue (PT/ST) was calculated as follows: PT/ST=Palisade tissue length /Spongy tissue length (Yao *et al.,* 2017).

Stomatal traits

For epidermal studies, leaves were soaked in absolute alcohol for 24 hours and then transferred to 80% acetone for 2-4 hours following the previous method of Tran *et al.* (2013). The leaf samples were immersed in NaOH/ethanol (1:5 mM NaOH/absolute ethanol). Next, the samples were placed on a glass slide with lactic acid and kept overnight.

The cross section of leaves and the stomatal microphotographs were taken using an electron microscope (Nikon Eclipse 80i, Japan) coupled to a digital microscope camera and filar micrometer. We analyzed 15 images per leaf, one leaf per plant, and three plants per treatment. Images were processed and analysed with ImageJ software (National Institutes of Health, USA). The size and density of stomata and epidermal cells were calculated for both the upper and lower epidermal surfaces.

Statistical analysis

Statistical analyses were conducted with Excel-software and R-software. Data were analyzed by analysis of variance (ANOVA), and differences between the means were tested using Ducan's test ($P < 0.05$).

Results

Growth parameters

Different intensities varied widely for the growth of spinach hydroponics. For the red – blue LED, the plant height and leaf number increased with increasing intensity. Table 1 shows that plant height and leaf number of spinach was higher in I3 (183 µmol/m2/s) and I4 (226 µmol/m2/s) than in I1 and I2. The difference was significant statistically. Specifically, plant height in I3 was higher 1.47; 1.18 times than in I1 and in I2, respectively. Plant height in I4 was higher corresponding 1.51; 1.21 times than in I1 and in I2. However, the difference in height and leaf number between I3 and I4 was not statistically significant.

Meanwhile, the leaf area index (LAI) was highest in I3 and decreased in the order of I3> I4> I2>I1. There are significant differences between 4 treatments at the 5% significance level. Besides, the RGR and NGR were highest in I3, but they were not significant difference in I2 and I4 (Table 1).

Table 1. Effect of different light intensities on plant height, leaf number of hydroponic cultivated spinach under a combination of red and blue LEDs in house (21 DAT)

Intensity of light treatment	Plant height (cm)	Leaf number (leaves/plant)	Leaf area (dm^2/plant)	RGR (g/day)	NAR (g/m^2/day)
I1(90 µmol/m2/s)	20.10^{c}	11.22^{b}	3.65^{d}	0.121^{b}	5.249^{c}
I2(140 µmol/m2/s)	24.95^{b}	12.44^{ab}	4.09^{c}	0.123^{b}	5.304^{b}
I3(190 µmol/m2/s)	29.60^{a}	13.33^{a}	5.85^{a}	0.138^{a}	5.465^{a}
I4(240 µmol/m2/s)	30.40^{a}	13.77^{a}	4.89^{b}	0.127^{b}	5.331^{b}
CV%	*2.6*	*6.6*	*4.6*	*3.8*	*0.5*

Different lowercase letters in the same column indicated significant differences among treatments (P≤0.05; n=3). The same as below. Net Assimilation Rate (NAR), Relative Growth Rate (RGR), DAT: days after transplanting.

Photosynthetic parameters and photosynthetic pigments

There was not statistically significant difference in the Chl*b* content and Chl*a*/Chl*b* of hydroponic spinach among the treatments, but the Chl*a* and Chl(*a*+*b*) contents were significantly higher in I3 treatment than in I1 and I2 treatments. The Chl*a*, Chl(*a* +*b*) and carotenoid contents did not differ significantly in the I1 and I2 treatments. Although, there was not statistically significant difference in Chl(*a*+*b*) and carotenoid contents between the I3 and

I4 treatments but they differed in Chl*a*. Whereby, the SPAD between the different treatments are statistically different (Table 2).

The Fv/Fm value and net photosynthesis rate (Pn) were highest in I3 treatment, that were significant different from other treatments. Specifically, Fv/Fm value in I3 treatment was 1.14; 1.13 and 1.11 times corresponding in I1, I2 and I3 treatments. But the Fv/Fm value did not differ significantly between in the I1, I2 and I3 treatments. There was also no statistically significant difference to Pn in I2 and I4 treatments (Table 3).

Table 2. Effect of different light intensities on photosynthetic pigments of hydroponic cultivated spinach under a combination of red and blue LEDs in house (21 DAT)

Intensity of light treatment	Chla (mg/g)	Chlb (mg/g)	Chl(a+b) (mg/g)	Chla/ Chlb	Carotenoids (mg/g)	SPAD
I1(90 µmol/m2/s)	0.256^{c}	0.481^{a}	0.738^{b}	0.532^{a}	0.161^{b}	32.31^{d}
I2(140 µmol/m2/s)	0.262^{bc}	0.495^{a}	0.757^{b}	0.528^{a}	0.167^{b}	34.98^{c}
I3(190 µmol/m2/s)	0.291^{a}	0.525^{a}	0.817^{a}	0.554^{a}	0.185^{a}	40.13^{a}
I4(240 µmol/m2/s)	0.276^{b}	0.507^{a}	0.783^{ab}	0.544^{a}	0.177^{ab}	37.22^{b}
CV%	*4.02*	*8.04*	*5.62*	*7.95*	*7.78*	*1.7*

Table 3. Effect of different light intensities on photosynthetic capacity of hydroponic cultivated spinach under a combination of red and blue LEDs in house (21 DAT)

Intensities of light treatment	Fo	Fm	Fv/Fm	Fv/Fo	Gs (mmol/ m^2/s)	Pn (µmol CO_2 /m^2leaf/s)
I1(90 µmol/m2/s)	0.120	0.606	0.802^{b}	4.05	0.24	32.48^{c}
I2(140 µmol/m2/s)	0.123	0.632	0.806^{b}	4.14	0.17	35.77^{b}
I3(190 µmol/m2/s)	0.044	0.504	0.912^{a}	10.45	0.10	44.26^{a}
I4(240 µmol/m2/s)	0.095	0.539	0.823^{b}	4.67	0.19	36.16^{b}
CV%			2.2			2.4

Stomatal conductance (Gs), Net photosynthesis rate (Pn).

Leaf anatomical features

Growth at higher intensity resulted in a 1.4 fold increased in leaf thickness from 259.10µm for I1 treatment leaves to 356.60 µm for I3 treatment leaves (Fig. 1; Table 4). This was due to a 1.3- 1.4 fold increased in the mean lengths of both the spongy mesophyll and the palisade cells. Furthermore,

differences in leaf thickness, palisade tissue length and spongy tissue length were statistically significant between 4 treatments. Whereas the PT/ST ratio was only different in I3 to I2, I4 and I1, but there was no significant difference between I2 and I4 treatments. Althrough, leaf compactness was not difference between 4 treatments (Table 4, Fig. 1). We did not notice the clear characteristic structure of the number of layers between 4 treatments, however the arrangement of palisade cells was compact and tidy with higher intensities. The spongy tissue cells were also distributed in an orderly and compact manner. There was more clearly about 2-3 layers palisade cells in I3 treatment.

Table 4. Effect of different light intensities on anatomical structure of hydroponic cultivated spinach leaves under a combination of red and blue LEDs in (21 DAT)

Intensities of light treatment	Palisade tissue length (µm)	Spongy tissue length (µm)	Leaf thickness (µm)	PT/ ST	Leaf compactness
I1(90 µmol/m2/s)	51.68^{d}	175.71^{d}	259.10^{d}	0.294^{c}	0.199^{a}
I2(140 µmol/m2/s)	60.02^{c}	196.85^{c}	298.45^{c}	0.305^{b}	0.201^{a}
I3(190 µmol/m2/s)	72.25^{a}	220.79^{a}	356.60^{a}	0.327^{a}	0.203^{a}
I4(240 µmol/m2/s)	65.23^{b}	210.04^{b}	324.33^{b}	0.311^{b}	0.201^{a}
CV%	*2.55*	*1.84*	*1.59*	*3.36*	*3.04*

PT: palisade tissue; ST: spongy tissue

Growth at higher intensity resulted in a 1.4 folds increased in leaf thickness from 259.10µm for I1 treatment leaves to 356.60µm for I3 treatment leaves (Fig. 1; Table 4). This was due to a 1.3- 1.4 folds increased in the mean lengths of both the spongy mesophyll and the palisade cells. Furthermore, differences in leaf thickness, palisade tissue length and spongy tissue length were statistically significant between 4 treatments. Whereas the PT/ST ratio was only different in I3 to I2, I4 and I1, but there was not significant difference between I2 and I4 treatments. Althrough, leaf compactness was not difference between 4 treatments (Table 4, Fig. 1). We did not notice the clear characteristic structure of the number of layers between 4 treatments, however the arrangement of palisade cells was compact and tidy with higher intensities. The spongy tissue cells were also distributed in an orderly and compact manner. There was more clearly about 2-3 layers palisade cells in I3 treatment.

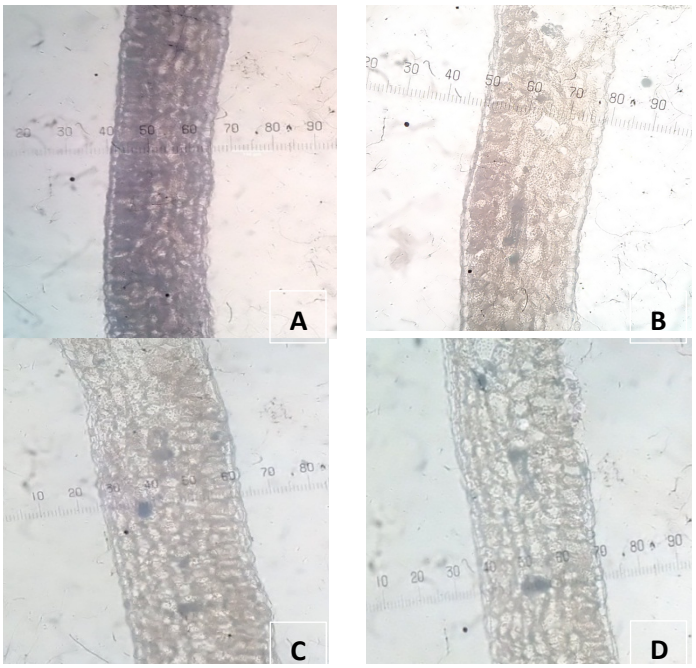

Figure 1. Effect of different light intensities on leaf anatomical structure of Spinacia oleracea L. (A-D, anatomy of leaf in A: 90 µmol/m2/s, B: 140 µmol/m2/s, C: 190 µmol/m2/s, D: 240 µmol/m2/s. Scale bar is 50 µm. The same as below.)

Stomatal traits

When light intensity increased, epidermal cell area, stomatal length, stomatal width increased. In the adaxial leaf surface and abaxial leaf surface, the epidermal cell area was highest in I3 treatment. But in all two leaf surfaces stomatal width was highest in the I4 treatment. The difference was statistically significant, except the epidermal cell area and stomatal width in abaxial leaf surface in I1 and I2 treatment; stomatal width in adaxial leaf surface in I2 and I3 treatments were not significant difference (Table 5, Fig. 2 and Fig. 3).

It can be seen that the higher the light intensity, the wider the guard cell. However, the length/width ratio of stomatal cells is highest in I3 treatments. Stomatal density was a statistically significant difference between treatments, however, the density increases as the intensity increases, which were not normal.

It was noted that stomatal density was highest in the adaxial leaf surface in I3 treatments but abaxial leaf surface was the highest density in I4

treatments. But, if we added the total number of stomatal cells in both leaf surface, they would be completely increased when the intensity of light increased (in order 218.84, 243.52, 328.97, 358.95 respectively I1, I2, I3, I4 treatments) (Table 5, Fig. 2 and Fig. 3).

Table 5. Effect of different light intensities on epidermal cells of hydroponic cultivated spinach leaves under a combination of red and blue LEDs in house (21 DAT)

Location	Intensities of light treatment	Epidermal cell area (μm^2)	Stomatal length (μm)	Stomatal width (μm)	Ratio of stomatal length:width	Stomatal density (number stomata/mm^2)
Adaxial leaf surface	I1	18.26^{d}	19.44^{d}	15.76^{c}	1.23^{c}	140.30^{c}
	I2	26.55^{c}	26.14^{c}	20.51^{b}	1.21^{c}	153.90^{b}
	I3	46.64^{a}	37.59^{a}	21.61^{b}	1.74^{a}	207.17^{a}
	I4	34.55^{b}	35.24^{b}	25.72^{a}	1.37^{b}	155.80^{b}
	$CV_{\%}$	*12.29*	*6.35*	*6.70*	*8.43*	*3.32*
Abaxial leaf surface	I1	18.29^{c}	21.73^{d}	17.54^{c}	1.240^{c}	78.54^{d}
	I2	19.84^{c}	24.78^{c}	18.18^{c}	1.365^{b}	89.62^{c}
	I3	31.74^{a}	36.61^{a}	19.91^{b}	1.838^{a}	121.80^{b}
	I4	24.47^{b}	27.66^{b}	25.34^{a}	1.095^{d}	203.15^{a}
	$CV_{\%}$	*12.13*	*5.56*	*6.28*	*7.95*	*4.47*

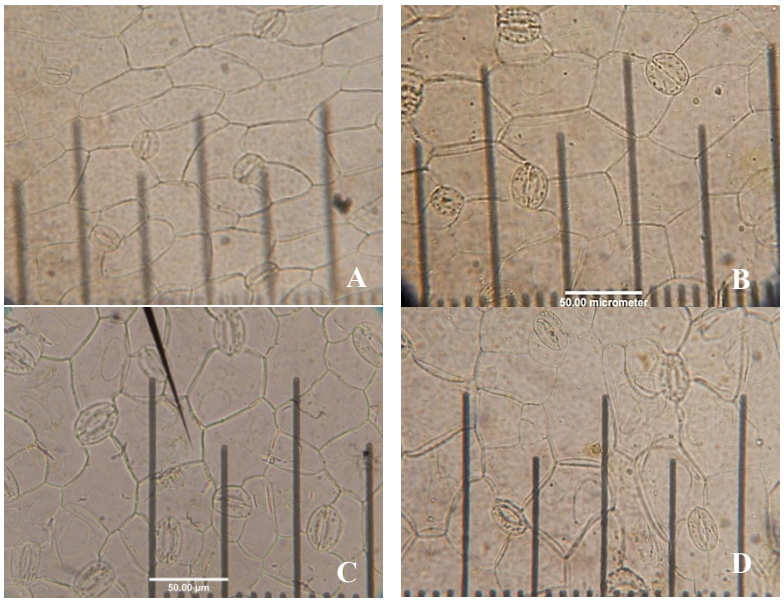

Figure 2. Effects of different light intensities on epidermal traits in spinach plants leaves (Adaxial leaf surface)

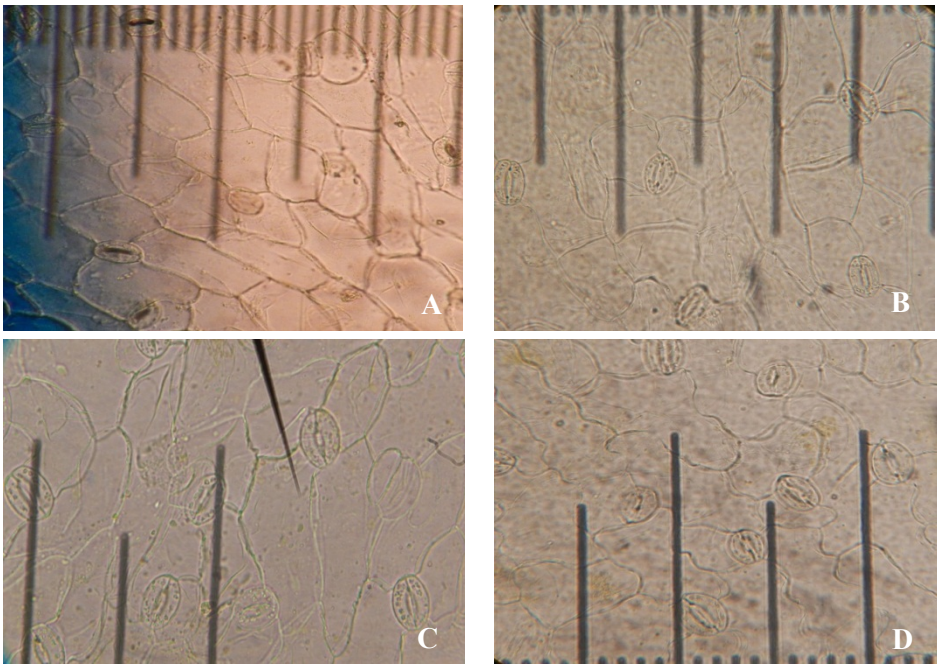

Figure 3. Effects of different light intensities on epidermal traits in spinach plants leaves (Abaxial leaf surface)

Table 6. Effect of different light intensities on yield of hydroponic cultivated spinach under a combination of red and blue LEDs in house (21 DAT)

Light treatment	Fresh weight of stem and leaf (g/plant)	Dry weight of stem and leaf (g/plant)	Theoretical yeild (kg/m^2)	Final harvest yeild (kg/m^2)
I1(90 µmol/m2/s)	16.35^{d}	0.92^{d}	1.37	1.28^{d}
I2(140 µmol/m2/s)	21.19^{c}	1.20^{c}	1.78	1.62^{c}
I3(190 µmol/m2/s)	43.74^{a}	1.64^{a}	3.67	3.46^{a}
I4(240 µmol/m2/s)	34.79^{b}	1.36^{b}	2.92	2.73^{b}
CV%	*2.7*	*5.85*	-	*3.1*

Productivity

Although the light intensities were increased but fresh weight and dry weight of stem and leaf, theoretical yield, final harvest yield were not highest in I4 treatments but in I3 treatments. The difference was the statistical significance between treatments. In I3 treatments fresh weight of stem and leaf, final harvest yield were 2.7 times higher than in I1treatment but dry weight of stem and leaf was only 1.8 times higher. Similarly, the fresh weight of stem and leaf, final

harvest yield in I4 treatment compared to in I1treatment were 2.1 times higher, but dry weight of stem and leaf was only 1.5 times higher (Table 6). This was suggested that higher yielding crops in addition to greater dry matter accumulation may be due to the large reserved of water in the foliage.

Discussion

Plant growth and development strongly depend on environmental factors. Among these factors, the light intensity is crucial. In this study, plant height, leaf development also as growth rate of hydroponic spinach (Tables 1) were enhanced with light intensities from 90-240 µmol/m^2/s provided by red and blue LEDs. Among these interval intensities, 190 µmol/m^2/s light intensity was the best effect for growth of hydroponic spinach. Photosynthetic pigments play the main role in photosynthesis. Chlorophyll molecules absorb light photons and switch to excited state then these excited chlorophyll molecules can dispose of light energy as photochemistry. Meanwhile, carotenoid molecules work as photoprotective agents by rapidly quenching the excited state of chlorophyll (Taiz and Zeiger, 2003). In our research, both Chl *a* and *b* contents in leaves increased under higher light intensity and highest in I3 treatment also as SPAD index and carotenoid (Tables 2). Althrough there was not different between Chl *a/b* in 4 treatments. The results were not whole similar to the results reported elsewhere, such as research of Yao *et al.* (2017) in rape seedlings. Under higher light intensity, plants accumulate more photosynthetic pigments to absorb more light energy. Moreover, under the limited supply of light energy (90 µmol/m^2/s), the Pn and Fv/Fm of hydroponic spinach were the lowest and this treatment also had the smallest chl*a* content (Table 2). The lasted researches indicated that if the leaves had the same thickness, larger leaves can capture more light energy to change photochemitry, so that leading to increase in biomass (Li and Kubota, 2009). The largest leaves and thickness of spinach in the I3 treatment explained why their dry weight was greater in the former than other treatments.

Plant leaf structure is influenced by environmental factors. Many researches showed that there is a flexible change before the change of external conditions, especially under the influence of light. The parameters such as thickness of the leaf, palisade tissue, and spongy tissue and the PT/ST in *Radix bupleuri* were reduced under shade conditions. These adaptive traits have also been reported in previous studies for *Datura stramonium* (Qin *et al.* 2014) and *Brassica napus* L. (Yao *et al.*, 2017).

Palisade cells concentrate more chloroplasts than spongy cells; even the high chlorophyll content appears in the first layer that allows little transmission of the incident light to the leaf interior. Thus, more light always penetrates in

the first layer of palisade cells. However, between spongy cells have air spaces generated many interfaces between air and water that reflect and refract the light, thereby randomizing its direction of travel (Taiz and Zeiger, 2003). Consequently, larger mesophyll cells can increase the internal surface area of the leaf and can improve photosynthetic efficiency. There are possible for that, the basic of photosynthesis is the palisade tissue. Some reports indicated that blue and red light provide energy for photosynthesis in the cell layers near the upper leaf surface, whereas in underlying cell layers the energy for photosynthesis is provided by green light (Vogelmann and Han, 2000). In another study, rape plants with thicker palisade tissue showed a higher net photosynthetic rate (Yao *et al.,* 2017). In our study, spinach in I3 treatment had the highest leaves thickness and palisade tissues, leaf compactness, thereby in this had higher Pn than others.

Stomata are considered a gate for CO_2 diffuses from the atmosphere into leaves. The stomatal pore is the major point of resistance to CO_2 diffusion. Therefore modulation of stomatal apertures allows controlling water loss and CO_2 uptake in plant. In addition, the CO_2 concentrating mechanism allows the leaf to maintain high photosynthetic rates at lower Ci values, which require lower rates of stomatal conductance for a given rate of photosynthesis. Stomata on the leaf and CO_2 absorption are positive correlated, but Pn and Gs are negatively correlated when light irradiation is less than the saturated light intensity of photosynthesis (Yao *et al.,* 2017). Our results were not out of this rule. In this case, Gs was smallest and Pn was highest in I3 treatment, but higher light intensity in I4 treatment had higher Gs and lower Pn values compare to I3 treatment due to saturated light intensity in I4 (240 μmol/m^2/s) for spinach in our conditions experiment. These results in this study were also consistent with Yao *et al.* (2017) in stomatal characteristics, such as: stomatal density, stomatal lenghth and stomatal length/width. But there is only one minor difference in this study I3 intensity showed that optimal effect than higher light intensity in I4 treatment.

The development of factors related to photosynthesis in which development of leaves is the basis for enhancing the assimilation and accumulation of anabolic products in plants. As a result, individual productivity as well as actual productivity is increased. The results of our study were also consistent with previous studies in which used of mixed red: blue light can also increase the crop yield such as research of Dong *et al.* (2014) in wheat (*Triticum aestivum* L.) Li *et al.* (2016) in okra (*Abelmoschus esculentus*) and Sabzalian *et al.* (2014). In any case, when environmental conditions are controlled, the red light can act as a principal source to promote the dry mass and yield of vegetables. As noted above, the blue, red light and their diffferent

intensities control the rates of photosynthesis through the opening and/or closing of stomata and their effect on plant biomass or yield is not surprising. In our study, it was possible that the spinach was suitable for the light intensity which was less than 240 µmol/m^2/s, thus I3 intensity gave the highest yield in the tested intensities.

It is concluded that the results clearly demonstrated the plant height, leaf number, leaf area, NGR, NAR, photosynthetic pigment, photosynthetic capacity and productivity of hydroponic cultivated spinach changed to adapt to different light intensities. The palisade and mesophyll tissues in the leaves were thicker and epidermal cell area, stomatal length, and stomatal width increased when light intensity increased. More important, the leaf area, NGR, NAR, Chl*a* and Chl(*a*+*b*) contents even leaf thickness was significantly higher in 190 $\mu mol/m^2/s$ treatment than in 240 $\mu mol/m^2/s$ treatment. Although, the light intensities were increased but fresh weight and dry weight of stem and leaf, theoretical yield, final harvested yield were not highest in 240 $\mu mol/m^2/s$ treatment but in 190 $\mu mol/m^2/s$ treatment. It showed that, 190 $\mu mol/m^2/s$ light intensity may be suitable intensity for growth of hydroponic cultivated spinach.

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(Received: 11 October 2018, accepted: 15 December 2018)